66. (new) Process according to claim 65 wherein the stabilizer is a substance acceptable as component in parenteral products and selected from the group consisting of human serum albumen (HSA), peptides, amino acids or proteins and mixtures thereof.

67. (new) Process according to claim 58 wherein the virus is a wild-type, attenuated or recombinant virus.

68. (new) Process according to claim 67 wherein the Flavivirus is Yellow Fever virus.

69. (new) Process according to claim 67 wherein the Flavivirus is an attenuated Yellow Fever virus.

70. (new) Process according to claim 68 wherein the Yellow Fever virus is the YF17D virus strain and/or substrains thereof.--

REMARKS

Reconsideration is requested.

Claims 1-41 have been canceled, without prejudice. Claims 42-70 have been added. Claims 42, 43, 45-59 and 60-70 are based on claims 1-15 and 30-41, respectively. Claims 44 and 60 are based on claims 2 and 31, respectively. No new matter has been added.

The objection of claims 9 and 36 and the Section 112, second paragraph, rejection of claims 1-15 and 30-41 are moot in view of the above.

The pending claims are submitted to be definite and consideration of the following in this regard is requested.

The applicants respectfully submit the objected-to phrase "acceptable as a substrate for vaccine production" is definite. On page 30 of the specification the applicants describe that the cell culture to be infected by the virus may be any cell insofar as virus can replicate and which is acceptable as a substrate for vaccine production, in particular any cell type which produces interferon in which flaviviruses or recombinants might grow and the same procedure of reducing cell density may be applied.

Moreover, the phrase "acceptable substrate" and routinely used by those of ordinary skill in the relevant art, such as in the art of vaccine production. An acceptable substrate is any cell culture which may be used for vaccine production, i.e., any cell culture which is unable to transfer any gene that may lead to undesirable side effects. An example of a cell culture that may not be used as an acceptable substrate for vaccine production is HeLa cells. HeLa cells may transfer oncogenes that may lead to the development of tumors.

Finally, the Examiner is requested to see the attached copy of U.S. Patent No. 6 306 637 as an example of a U.S. patent that uses the objected-to phrase in the claims. The present Examiner is not believed to be suggesting that the claims of the attached patent are invalid or indefinite for the use of this phrase.

The claims are submitted to be definite in this regard.

The applicants respectfully submit that the objected-to phrase "suitable medium" is definite and consideration of the following in this regard is requested.

The phrase "suitable medium" is well known by those of ordinary skill in the art of vaccine production, as medium comprising mainly amino acids, vitamins and mineral salts. The Examiner is requested to see the attached copy of U.S. Patent No. 6 194 210 as an example of U.S. patents that use this type of language in their claims. The objected-to phrase is understood by those of ordinary skill in the art.

The claims have been amended to include antecedent basis for subsequently references cell cultures. The claims are submitted to be definite in this regard.

The phrase "appropriate period of time" will be recognized as embracing periods of time which are appropriate for the incubation of the cell cultures of the present invention. In addition, it comprises ranges of temperatures which are commonly known and used by those skilled in the art. Contrary to the Examiner's assertions, the phrase is recognized by one of ordinary skill in the art.

The phrase "once or more times" means that this step of washing shall be repeated until those skilled in the art consider the cells are free of undesirable components. The metes and bounds of this phrase will be recognized by one of ordinary skill in this art.

The claims have been amended with regard to inclusion of the article "a" prior to the recitation of a stabilizer.

The phrase "harvesting of culture supernatant virus with or without addition of a stabilizer" may be understood by the specification (pages 4, second paragraph, 10, two

paragraphs before example 1, and example 3). The following passages of the specification are supportive:

..." Whether stabilizer is used, it may be added to the virus harvested at steps with medium changing prior to storage at –45º C to –196º C..." (page 5, lines 41-42 of the specification);

..." Viruses are recovered after incubation by centrifugation or filtration to remove cellular debris. To the supernatant containing the virus a stabilizer was added which is, for those skilled in the art, known to enhance the stability of viral infectivity during freezing, thawing and subsequent manipulations..." (page 14, lines 27-30 of the specification).

One of ordinary skill in the art would understand the objected-to phrase and the claims are submitted to be definite in this regard.

The applicants respectfully submit that that the phrase "removing cells debris and whole cells from the harvested virus" does not mean that complete or integral cells are removed from the harvested virus, i.e., the term "whole" as used in now-canceled claims 1 and 30 means cells which were not disrupted.

The applicants respectfully submit that means of viral inactivation are well known and the purpose of the same are well understood. The Examiner is urged to appreciate that vaccines can be produced from either weakened or dead viruses. Concerning dead viruses, the killing process is a procedure well known by those ordinarily skilled in the art as, for example, virus or viral inactivation. After the virus is inactivated, it is no longer able to replicate and produce disease in the body. However, after injection it is

still able to stimulate an immune response. Viruses are "killed" by using chemical agents (e.g. formaldehyde) and the resultant vaccines are known as inactivated virus vaccines. Those ordinarily skilled in the art will know how and when to perform viral inactivation of a given virus. The claims are definite in this regard.

The applicants respectfully submits that the objected-to phrase "when submitted to viral infection" is related to the production of interferon by certain types of cells which have been stimulated by exposure to a virus (Page 3, third to ninth paragraphs). In the production of vaccine viruses in cell cultures, the interferon system is responsible for low virus yields, but the present invention is related to a process that have promoted these virus yields according to it was shown in the Detailed Description of the Invention and in its Examples.

The applicants recitation of "any further passaged" will be understood by one of ordinary skill in the art. The phrase will be recognized as referring to the culture of cells which is transfected with the virus for the first time or resulting from subsequent transfections. The pending claims are submitted to be definite in this regard. In addition, the Examiner will appreciate that this is a common technique used to obtain attenuated vaccine viruses as described in the second paragraph of the Background of the Invention. One of ordinary skill in the art will appreciate how to obtain further passaged cultures of cells.

The claims have been amended with regard to the recitation of "steps" and the Examiner's objection thereof. The pending claims are submitted to be definite in this regard.

The applicants urge the Examiner to appreciate that "parenteral products" are recognized by those of ordinary skill in the art as products which are not administered by digestive tube, e.g. intramuscularly or subcutaneously. What makes a component acceptable and what criteria is used to make such an evaluation may be understood by the specification (page 11, penultimate paragraph and page 12, second paragraph), such as from the following passages:

"... Another relevant aspect in vaccine virus production is related to materials used in the culture medium since foreign proteins are not acceptable as components of human vaccines. For example, fetal bovine serum proteins are not acceptable in parenteral products in view of their immunogenicity. On the other hand, tests have shown that low virus yields from cell cutures, e.g. CEF, are obtained in the absence of fetal bovine serum (FBS) ...

...

...Therefore, according to the invention, medium supplements acceptable as components in parenteral products are used in cell cultures to stabilize the virus produced in order to obtain high virus yields, e.g. human serum albumen (HSA). Other substances acceptable as components in parenteral products, such as peptides, amino acids, proteins, can be used..."

One of ordinary skill will also appreciate the metes and bounds of the objected to phrase such that the claims are submitted o be definite in this regard.

The claims have been amended to refer to "wild-type" to obviate the Examiner's objection to the previous recitation of "wild".

Finally, with regard to the Examiner's objections of now canceled claims 15 and 41, the applicants note the claims refer to a procedure for the propagation of 17D viruses in general, being the 17D virus derived, for example, from further passaging of any existing 17D substrain (page 8, sixth paragraph after Table III). However, as known by a person of ordinary skill in the art, a seed lot of YF17D virus can also comprise a mixed population of this virus because commonly it has not been purified before the subsequent passagings. So this mixed population can be used since a virulent phenotype is not present. The claims have been amended in this regard to reflect the same and the pending claims are submitted to be definite.

The Section 102 and Section 103 rejections of the claims over Barrett, A.D.T., Monath, T.P., Cropp, C.B., Adkins, J.A, Ledger, T.N., Gould, E.A, Schlesinger, J.J., Kinney, R.M. and Trent, D.W. *Attenuation of Wild- Type Yellow Fever Virus by Passage in HeLa Cells.* Journal of General Virology, 71: 2301-2306 (1990) are moot in view of the above. The claims are submitted to be patentable over the cited art and the Examiner's consideration of the following in this regard is requested.

The applicants submit that Barret *et al* were interested in the molecular basis of attenuation and virulence of Yellow Fever virus. To analyze attenuation/virulence of this virus, these researches repeated and observed the passage of YF virus in HeLa cells. In addition, they repeated the experiments using monoclonal antibodies, identifying changes in the viral envelope protein that may related to virus virulence.

However, the applicants have developed an improved process to produce interferon inducing/sensitive vaccine viruses, being characterized by an increase in the growing virus yields.

In addition, it is known by one of ordinary skill in the art of vaccine production, for example, that regardless of how the vaccine virus is obtained by inactivation, attenuation or the use of recombinant DNA techniques, the virus must be compatible with the host cell, i.e., capable of autonomous replication in the host cell. Moreover, in the large-scale production, the host cell must be a cell insofar as the virus can grow with high yield. These cells are infected with the virus ("seed virus") which then replicates in the cell and is usually released into the culture medium. The conditions and materials used in the whole process must be carefully monitored to avoid problems mainly related to: genetic variability, loss of immunogenicity, virus inactivation, neurovirulence, low yield in relation to virus input and contamination by extraneous agents.

The applicants also note that cells for the production of virus vaccines for parenteral use in man are limited to those which are free of adventitious agents, non-tumorigenic and karyologically normal. HeLa cells (derived from carcinoma of human uterine cervix) is an example of a cell culture that may not be used as an acceptable substrate for vaccine production because these cells may transfer oncogenes that may lead to the development of tumors.

The cited art therefore does not teach or suggest the presently claimed invention.

In view of the above and attached, the claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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By: _____

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